

TITLEREAL TIME QUANTITATIVE PCR WITH INTERCALATING DYE FOR SINGLE AND  
MULTIPLEX TARGET DNA

## ABSTRACT

5           The PCR-based, dsDNA quantification method monitors the fluorescence of a target, whose melting characteristics is predetermined, during each amplification cycle at selected time-points. Fluorescence is measured immediately after the annealing phase ( $F_E$  at  $T_E$ ), immediately below ( $F_{MS}$  at  $T_{MS}$ ) and above ( $F_{ME}$  at  $T_{ME}$ ) the melting of the target/amplicon. A change in slope from a baseline slope ( $S_B = -(F_{MS} - F_E)/(T_{MS} - T_E)$ ) to a  
10   melting phase slope ( $S_M = -(F_{ME} - F_{MS})/(T_{ME} - T_{MS})$ ) indicates a specific amplification. The number of amplification cycles ( $C_T$ ) it takes for the quantity ( $S_M - S_B$ ) to become greater than zero correlates with the starting concentration of the target ( $C$ ). The concentration of the target in a sample is determined by comparing the value of  $C_T$  for the sample with a standard curve. By selecting targets with distinguishable melting curve characteristics,  
15   multiple targets can be simultaneously detected.

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